

Risk Factors Associated With Hepatitis C Virus (HCV) Infection Among Prostitutes and Their Clients in the City of Santos, São Paulo State, Brazil

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We studied the role of sexual transmission in the epidemiology of HCV by a cross-sectional study comparing prostitutes and HCV seropositive and seronegative sexual clients recruited from the bordellos of the docks of Santos, São Paulo State. The average age in the prostitute group was 27.2 years. The median time spent in prostitution was 3 years. The average number of clients per week was 7. A total of 5.2% of the prostitutes admitted to having used injectable drugs. Nine percent patients said that they had received a blood transfusion and 36.3% claimed to have had a sexually transmissible disease in the past. The prevalence of HCV antibodies was 10.9%. There was a positive and independent relationship between HCV seropositivity and the following variables: use of injectable drugs (OR = 5.2; 95% CI = 2.2 to 12.2), prior blood transfusion (OR = 2.3; 95% CI = 1.08 to 4.9), time spent in prostitution (OR = 2.0; 95% CI = 1.13 to 3.6), and a positive FTA-ABS result (OR = 1.7; 95% CI = 0.95 to 3.0). The risk factors indicating parenteral exposure (use of an injectable drug and prior blood transfusion) presented a stronger relationship with HCV seropositivity. The time spent in prostitution and FTA-ABS positivity, risk factors indicating sexual exposure, also presented a positive relationship with HCV seropositivity, suggesting a significant role for sexual transmission in HCV epidemiology, particularly in groups involved in promiscuous sexual behavior. *J. Med. Virol.* 51:338–343, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: prostitution; sexually transmitted diseases; hepatitis C virus

INTRODUCTION

The possibility of sexual transmission of the agents causing non-A non-B hepatitis (H-NANB) had been considered before the identification of HCV [Alter et

al., 1989]. The cloning of the HCV genome and the development of an immunoenzymatic assay enabled a more comprehensive study to be made of HCV epidemiology [Alter et al., 1989a; Bradley et al., 1992; Choo et al., 1989; Kuo et al., 1990]. Studies of individuals subject to frequent parenteral exposure (intravenous drug user and hemophiliacs) showed a uniformly high prevalence of HCV [Esteban et al., 1989; Fairley et al., 1990; Bell et al., 1990; Van den Hoek et al., 1990; Vanderschueren et al., 1991; Zeldis et al., 1991]. Studies investigating sexual transmission, for example, among prostitutes, male homosexuals, and STD clinic patients gave contradictory results. The majority of authors were unable to detect a significant association between HCV infection and variables indicating greater sexual exposure [Tor et al., 1990; Brettler et al., 1990; Everhardt et al., 1990; Melbie et al., 1990; Kolho et al., 1991; Shev et al., 1991; Van Doornum et al., 1991; Gordon et al., 1992; Brackmann et al., 1993; Bresters et al., 1993]. Some reports, however, suggested, that sexual transmission of HCV could be greater than had been anticipated [Tedder et al., 1991; Kao et al., 1992; Kihara et al., 1993; Nakashima et al., 1993; Orduña et al., 1992; Wu et al., 1993].

In this study, we investigated the prevalence and risk factors associated with HCV antibody seropositivity among prostitutes and their clients in an attempt to understand the relative role of sexual transmission in this group.

MATERIALS AND METHODS

Setting and Patients

This was a cross-sectional study investigating the seroprevalence of HCV and other sexually transmitted diseases in prostitutes and their clients living in the

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city of Santos, Brazil. Socio-demographic, behavioral, and medical data were collected through a validated questionnaire applied by trained interviewers between 1986 and 1990. Santos is located on the southern sea-coast of the State of São Paulo. A vast red-light district extends in close proximity to the port. At the worksite, prostitutes and their clients were approached and invited to participate in the study, give a sample of blood, and answer a questionnaire. Over 90% of the prostitutes approached agreed to participate. Each participant was enrolled only once during the 3-year period of the study. Serum samples were tested for antibodies against recognized sexually transmissible agents, including HIV-1, HIV-2, HBsAg, HTLV-1, HTLV-2, and *T. pallidum* at the State Reference Laboratory in Santos. The operational definition of prostitutes was a woman that exchanged sexual favors for money or other material assets within the site under study. Men who paid the prostitutes for occasional sexual favors were considered to be clients. Only prostitutes and clients were studied. Individuals who admitted the use of intravenous drugs in the last year were regarded as being injecting drug users. Drug use by other routes was not considered. Condom use was defined as its use in the majority of cases of sexual intercourse. Given the cross-sectional nature of the study, we did not feel confident in estimating the lifetime number of sexual partners. An analysis was performed on samples from 686 prostitutes and their clients, gathered in June 1987, December 1988, and June 1990.

Serological Techniques

Detection of HCV antibodies was performed by second-generation immunoenzymatic assay, which demonstrates antibodies against the nonstructural c200 and c33-c antigens, and against the structural c22-3 antigen (Monolisa anti-HCV, Sanofi, Diagnostics Pasteur, France). Repeat positive samples underwent confirmation by the recombinant immunoblot technique. This technique detects antibodies against the non-structural c100-3, c5-1-1, and c33-c antigens, and structural C22-3 (Riba-Deciscan, Sanofi, Diagnostics Pasteur, France). Reaction to at least 2 of these antigens was necessary before a result was regarded as positive (with the exception of superoxide dismutase SOD). Samples that presented reactivity to only one antigen or SOD were regarded as indeterminate. Samples that failed to react to any antigen were regarded as negative. These assays were performed by the Special Immunovirology Laboratory, Discipline of Infectious and Parasitic Diseases, Federal University of São Paulo. HIV-1 and HIV-2 serology was performed by application of first-generation immunoenzymatic assay (Biolab Diagnóstica, SA, Brazil). Assays were performed in compliance with the manufacturer's instructions. Repeat positive samples underwent a Western blot-type assay (Du Pont de Nemours, US, and Biolab Diagnóstica, SA, Brazil). The treponemal assay, after absorption of the Reiter-strain serums and readout by fluorescent microscope, was employed for specific *T.*

TABLE I. Characteristics of the Prostitutes

| Characteristic | Range | Median | SD |
|-------------------------------------|-------|--------|------|
| Age ^a | 14–58 | 26.0 | 8.1 |
| Time in the profession ^a | <1–36 | 3.0 | 5.4 |
| Number of clients ^b | 1–70 | 7.0 | 11.2 |

^aIn years.

^bPer week.

pallidum antibodies. Screening for the HBV surface antigen (HBsAg) was performed by the immunoenzymatic technique, employing monoclonal antibodies. Screening of HTLV-I and -II antibodies was performed by means of immunoenzymatic assay (Cambridge Bioscience, US). Repeat positive samples were tested by the Western Blot for HTLV-1 (Imunoblot Embrabio, São Paulo; Biotin US; Genetic System, US). Criteria for performing and interpreting results were in accordance with manufacturers' instructions.

Statistical Analysis

Statistical analysis was undertaken using the 2D, 4F, and LR programs of the BMDP (1992 version) statistical package. These programs perform the chi-square tests, including the Pearson and Yates corrections, the Mantel-Haenszel chi-square test for bivariate analyses, and logistic regression. Fischer's exact test was employed to compare frequencies in which more than 20% of the expected values below 5.

RESULTS

The average age in the prostitute group was 27.2 years. The median of time spent working as a prostitute was 3 years. The average number of clients per week was seven (Table 1). Just over 5% of prostitutes admitted to the use of intravenous drugs during the preceding year, 9% had received a blood transfusion, and 36% said that they had at some time suffered from a sexually transmissible disease (Table 4).

Table 2 presents the results of the serological tests performed in the population studied. The prevalence of HCV antibodies was high, 15.3% when the ELISA method was employed and 10.9% with the RIBA. Just over 71% of the positive ELISA results were confirmed by RIBA. The prevalence of HIV-1 antibodies was 2.5%; for HTLV1, 2.0%; for *T. pallidum*, 29.8%; and for HBsAg 6, 9% (Table 3).

The following variables did not reveal a positive relationship with HCV seropositivity, using a second generation recombinant immunoblot assay: number of children, past history of STD, positive Western blot for HIV, frequency of anal intercourse, average number of clients per day, positive HBsAg, and positive Western blot for HTLV. Tables 4 and 5 lists the variables that were positively associated with the presence of HCV antibody, using univariate analysis in clients and prostitutes, respectively. These variables were then submitted to logistic regression and the results are shown in Table 6.

TABLE II. Result of Hepatitis C Serology by Laboratory Technique*

| Result | Technique | | | | | |
|----------|-------------|-------------|-------------|--------------|--------------|--------------|
| | ELISA | | | RIBA | | |
| | Clients | Prostitutes | Total | Clients | Prostitutes | Total |
| Negative | 127 (86.4%) | 454 (84.2%) | 581 (84.6%) | 129 (87.80%) | 467 (86.7%) | 596 (86.9%) |
| Positive | 20 (13.6%) | 85 (15.8%) | 105 (15.3%) | 17 (11.50%) | 58 (10.7%) | 75 (10.9%) |
| Indeterm | | | | 1 (0.07%) | 14 (02.6%) | 15 (02.2%) |
| Total | 147 (100%) | 539 (100%) | 686 (100%) | 147 (100%) | 539 (100.0%) | 686 (100.0%) |

*For statistical analysis, the negative results of ELISA and indeterminate ones of RIBA were considered negative for RIBA.

TABLE III. Seropositivity for Other Sexually Transmitted Diseases

| Agent | Positive result | | |
|---------|-----------------|-------------|-------|
| | Clients | Prostitutes | Total |
| HIV | 0.4% | 2.1% | 2.5% |
| HTLV | 0.2% | 1.8% | 2.0% |
| FTA-ABS | 3.5% | 26.3% | 29.8% |
| HBsAg | 1.1% | 4.9% | 6.0% |

TABLE IV. Variables Associated With a Positive HCV Status (RIBA-2 Second Generation Recombinant Immunoblot Assay) in Clients, in a Univariate Analysis

| Variable | HCV (+) | HCV (-) | Level significance (P) |
|-----------------------|---------|---------|------------------------|
| | n = 17 | n = 130 | |
| Age (mean) | 33 | 30 | 0.15 |
| Blood transfusion (%) | 0 | 5.4 | 0.32 |
| Partner drug user (%) | 41.2 | 15 | <0.001 |
| Non-use of condom (%) | 36.4 | 53.6 | <0.28 |
| Positive FTA-ABS (%) | 29.4 | 14.6 | <0.28 |

TABLE V. Variables Associated With Positive HCV Status (RIBA-2 Second Generation Recombinant Immunoblot) in Prostitutes, in a Univariate Analysis

| Variable | HCV (+) | HCV (-) | Level significance (P) |
|--------------------------|---------|---------|------------------------|
| | n = 58 | n = 481 | |
| Age (mean) | 26.11 | 29.01 | 0.09 |
| Blood transfusion (%) | 19.0 | 9.2 | 0.02 |
| Partner drug user (%) | 43.1 | 31.5 | 0.07 |
| Non-use of condom (%) | 70.0 | 85.1 | 0.03 |
| Positive FTA-ABS (%) | 46.6 | 3.19 | 0.02 |
| Prostitution time (mean) | 4.79 | 6.98 | 0.07 |

DISCUSSION

The high prevalence of HCV among prostitutes in Santos and the association between positive status and use of injectable drugs, prior blood transfusion, involvement in prostitution for over 5 years, and FTA-ABS positivity were the main findings of this study. The study suggests that sexual transmission may be important in the spread of HCV. Published data on the prevalence of HCV among the sexual clients of infected

TABLE VI. Odds Ratio (OR) and 95% Confidence Interval (95% CI) of Variables Associated With HCV Positive Status, in Prostitutes, After Logistic Regression

| Variable | Coefficient | Odds ratio | 95% CI | |
|-----------------------------------|-------------|------------|--------|-------|
| | | | Lower | Upper |
| Use of injectable drug | 0.192 | 5.2 | 2.2 | 12.2 |
| Blood transfusion ^a | 0.430 | 2.3 | 1.08 | 4.9 |
| Time in prostitution ^b | 0.493 | 2.0 | 1.13 | 3.6 |
| Positive FTA-ABS | 0.589 | 1.7 | 0.95 | 3.0 |

^aWithin the last five years.

^bMore than five years.

individuals and which partner and index case do not belong to the same risk group are contradictory, as can be seen in Table 7.

Prevalence of HCV among prostitutes in our study was high (10.9%). This rate contrasts with that for blood donors in Brazil, 2.2% [Marliere, 1993]. Seventy-one percent of cases identified as positive by ELISA were confirmed by RIBA, which is also high. The high rate of HCV found in prostitutes (15.3%) and their clients (13.6%) is higher than that reported in injecting drug users and hemophiliacs [Esteban et al., 1989; Fairley et al., 1990; Bell et al., 1990; Van den Hoek et al., 1990; Vanderschueren et al., 1991; Zeldis et al., 1991]. This comparison allows us to classify the population as at intermediate risk of HCV infection, suggesting that there is a possible association between HCV seropositivity and promiscuous sexual behavior.

Duration of involvement in prostitution was found to be an independent risk factor for HCV infection (OR = 2.0; 95% CI = 1.13–3.6), a finding confirmed by the work of Wu et al. [1993]. Nakashima et al. [1993] found an HCV prevalence 6 times higher among prostitutes who had been involved in the profession for more than 1 year. However, this difference did not achieve statistical significance ($P > 0.05$). Gutierrez et al. [1992] and Orduña et al. [1992] were unable to reproduce these results.

The prevalence of FTA-ABS seropositivity was high and turned out to be independently associated with HCV infection (OR = 1.7; 95% CI = 0.95 to 3.0), even after adjusting for use of intravenous drug use, blood transfusion, and time in prostitute. Gutierrez et al. [1992], Orduña et al. [1992], and Nakashima et al. [1993] also detected a statistically significant associa-

TABLE VII. Prevalence of Seropositivity Among Sexual Partners of HCV-Infected Individuals

| Author | Positive | N | Risk group/index case | Country |
|-------------------------|-----------|-----|-----------------------|-------------|
| Schulmann et al. (1990) | 00/ 0.0% | 13 | HCV + (hemophiliacs) | Sweden |
| Tor et al. (1990) | 16/11.0% | 143 | HCV + (drug users) | Spain |
| Shev et al. (1991) | 00/ 0.0% | 13 | HCV + (blood donors) | Sweden |
| Kolho et al. (1991) | 00/ 0.0% | 30 | HCV + (hemophiliacs) | Finland |
| Widell et al. (1991) | 00/ 0.0% | 12 | HCV + (hemophiliacs) | Sweden |
| Lee et al. (1991) | 02/ 5.9% | 34 | HCV + (blood donors) | Taiwan |
| Pachucki et al. (1991) | 01/ 0.04% | 25 | HCV + (drug users) | USA |
| Kao et al. (1992) | 07/18.4% | 38 | HCV + (blood donors) | Japan |
| Akahane et al. (1992) | 14/07.1% | 195 | HCV + (blood donors) | Japan |
| Zeldis et al. (1992) | 04/ 4.9% | 81 | HCV + (drug users) | USA |
| Breesters et al. (1993) | 00/ 0.0% | 50 | HCV + (PCR+) | Netherlands |

tion between FTA-ABS seropositivity and HCV infection in prostitutes. Surprisingly, in contrast to similar prevalence of HCV found among prostitutes and their clients, the prevalence of HIV, HBV, HTLV, and syphilis were strikingly higher among prostitutes. The reasons for such a discrepancy are not fully understood. We did not find any association between HCV infection and seropositivity for HIV, HBV, and HTLV/II viruses ($P = 0.4295$, $P = 0.7215$, $P = 0.7632$, respectively).

A clear association was found between injecting drug use and past history of blood transfusion and HCV infection (OR = 5.2 and 2.3; CI = 2.2–12.2 and 1.08–4.9; respectively), as expected. This finding has been replicated many times in the scientific literature [Van den Hoek et al., 1990; Zeldis et al., 1992; Melbie et al., 1990; Riestra et al., 1992; Tabet et al., 1992; Wu et al., 1993]. Van den Hoek et al. [1990] found a significant association between injectable drug use and HCV seropositivity (OR = 7.3; 95% CI = 2.3–23.6). This association does not seem to depend on the type of drug injected, on the frequency of use, or habits such as sharing of syringes and needles.

It is possible to classify the variables studied into 2 groups according to the type of exposure indicated: those indicating sexual exposure, and those indicating parenteral exposure. Univariate analysis revealed an association between HCV seropositivity and age, blood transfusion, injecting drug use, non-use of condoms, and time spent in prostitution. Variables indicating parenteral exposure showed a strong association with HCV seroprevalence ($P < 0.0001$ for injectable drug use and $P = 0.02$ for blood transfusion), as has been previously demonstrated [Esteban et al., 1989; Bell et al., 1990; Widdel et al., 1991; Zeldis et al., 1992].

Data was also found that suggested a role for sexual transmission of HCV. Associations were observed between HCV infection and age ($P = 0.0369$), time spent in prostitution ($P = 0.0033$), non-use of condoms ($P = 0.0033$), sexual partners habits ($P = 0.0089$), and FTA-ABS positivity ($P < 0.01$). The association between HCV infection and those variables indicating greater sexual exposure were studied after controlling for injecting drug use. The time spent in prostitution remained significantly associated with HCV infection for both injecting drug users and non-users ($P = 0.0284$).

This observation reinforces the independent nature of the association between the time spent in prostitution and HCV infection and is in accordance with the reports of other authors [Nakashima et al., 1993; Wu et al., 1993].

An independent association was also observed between HCV seropositivity, having spent more than 5 years in prostitution, and positive FTA-ABS. These variables indicate sexual promiscuity, suggesting that HCV may be sexually transmitted.

The basic conditions for the study of sexual HCV transmission are: (1) existence of a viremic index case, (2) occurrence of exposure, (3) conditions and type of exposure, and (4) occurrence of infection. A transmitter is regarded to be any HCV seropositive individual. Van der Poel et al. [1991] and Romeo et al. [1993] found a relationship between infectivity and HCV antibody seropositivity by means of the RIBA-2 method. However, seropositivity, according to the methods currently available, is not synonymous with infectivity. Breesters et al. [1993] performed a study in which they were able to bypass this limitation. Fifty sexual contacts of HCV seropositive individuals (ELISA-2 and RIBA-2), as well as the viremic individuals (PCR RNA-HCV+), were included in the study. None of the partners was seropositive. The author explains the absence of sexually transmitted HCV as being due to the low viral titers present in the serum and secretions of infected patients. It is important to point out the selection of index cases. This must be the starting point in studies about sexually transmitted HCV.

The second item relates to exposure. Here one needs to consider the frequency and type of sexual contact, the occurrence of STD with ulcerating lesions that can act as a port of entry, and mainly the HCV concentration in genital secretions. Breesters et al. [1993] found titers of HCV RNA lower than 625×10^5 genome equivalents/milliliter of blood among fifty HCV seropositive patients. Hsu et al. [1991] found similar results. For purposes of comparison, HBV, which has a high risk of sexual transmission, contains titers that range from 10^5 to 10^{10} genome equivalents/milliliter of blood and 10^5 to 10^6 in sperm and saliva [Jenison et al., 1987]. The infectious dose of HCV, both in vivo and in vitro, is unknown, but appears to be low. With HBV,

the viral concentration in sperm and other secretions is the limiting factor for sexual transmission. Other factors could make infection easier, even with a small inoculum, for example, ulcerating genital lesions or traumatic sexual practices that lead to a loss of the integrity of the mucous membrane. Consideration should also be given to the time required, in the event of infection, for the detection of antibodies by methods currently available and to how long they will be detectable. The development of more accurate diagnostic tests will afford a better understanding of the natural history of infection by hepatitis C virus.

Finally, it is necessary to consider the possibility of error in the analysis or in the gathering of data. The use of intravenous drugs, as well as other forms of parenteral exposure (tattooing, acupuncture, use of earrings, etc.) have been reported among individuals with promiscuous sexual behavior [Osmund et al., 1991; Stary et al., 1992; Bresters et al., 1993]. These authors suggest that these are the principal paths for infection in promiscuous individuals who live in areas endemic for hepatitis C. Therefore, the association we found between seropositivity and sexual promiscuity may be an artifact, caused by the possibility that sexual promiscuity is a marker for other risk behaviors. Furthermore, we may have underestimated the true prevalence of intravenous drug use. This could have occurred for 2 main reasons, first, because the interviewers did not feel at liberty to discuss their injecting behavior, or second, because intravenous drug use occurred outside of the time frame used in this study (within the last year).

The natural history of hepatitis C is different from that of other viral forms of viral hepatitis. Slow progressive development, low viremia, synthesis of non-neutralizing antibodies, and the high rate at which it becomes chronic places hepatitis C in a class by itself. It is natural to expect that its standards of transmission be different from those known at the present moment, as shown by the majority of surveys so far performed. The potential for sexual transmission of HCV is obviously lower than that of HBV, of other hepatotropic viruses, and of HIV. However, this does not allow us to conclude that sexual transmission is unimportant for maintenance of HCV in the human species.

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